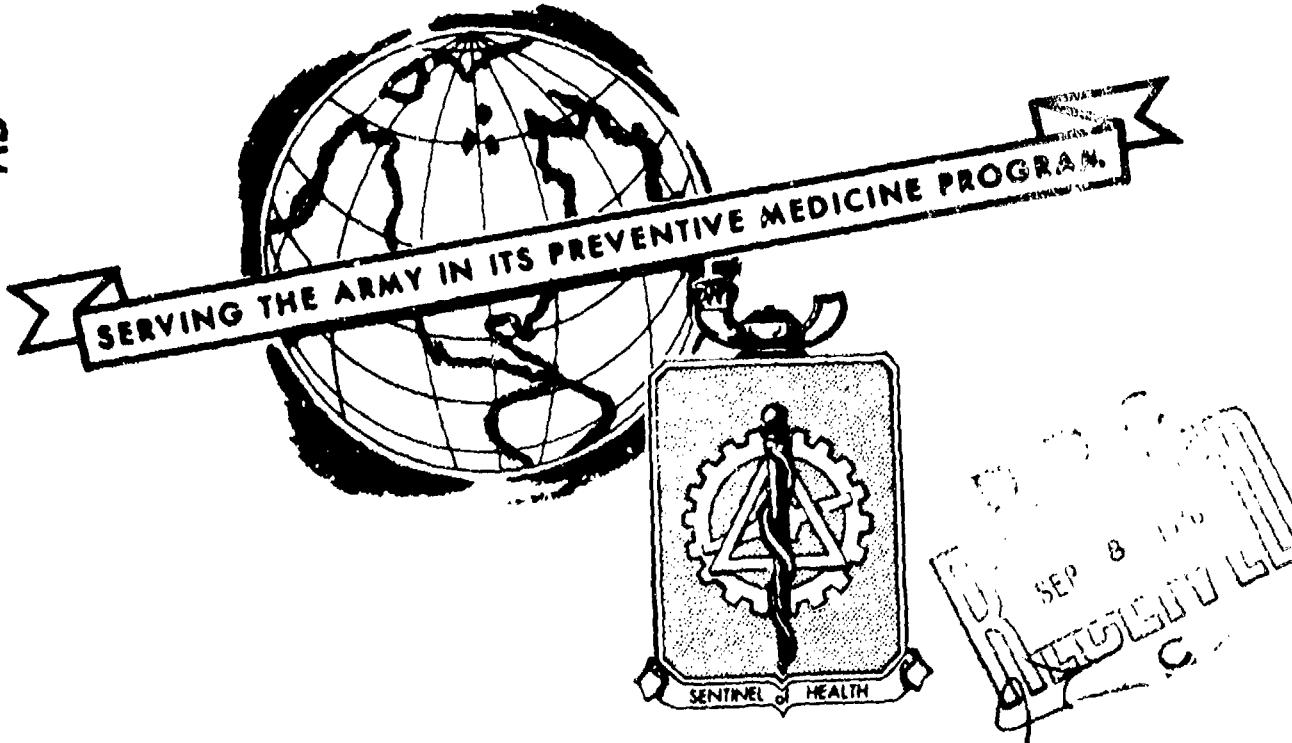


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US ARMY
ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MD 21010

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BEHAVIORAL AND BIOCHEMICAL
EFFECTS OF MALATHION
STUDY NO. 51-051-73/76
OCTOBER 1975 - APRIL 1976

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↓ produced significant decreases in blood and brain cholinesterase activity as well as avoidance performance. Spontaneous motor activity was not significantly changed at any dosage. The results suggest that low dosages of malathion may disrupt behavior without significantly reducing cholinesterase activity.

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HSE-LT/WP

3 SEP 1976

BEHAVIORAL AND BIOCHEMICAL
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ABSTRACT

Rat conditioned avoidance performance and spontaneous motor activity were examined after intraperitoneal injection with 25, 50, 100, or 150 mg/kg of the organophosphate insecticide, malathion. The effects of these dosages on plasma, erythrocyte, and brain cholinesterase activity were also assessed. Avoidance performance was significantly impaired 1 hour after injection with 50 mg/kg although blood and brain cholinesterase activity remained at greater than 90 percent of control values. The higher dosages (100 and 150 mg/kg) produced significant decreases in blood and brain cholinesterase activity as well as avoidance performance. Spontaneous motor activity was not significantly changed at any dosage. The results suggest that low dosages of malathion may disrupt behavior without significantly reducing cholinesterase activity.

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EFFECTS OF MALATHION
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OCTOBER 1975 - APRIL 1976

1. AUTHORITY. US Army Environmental Hygiene Agency Program Document, FY 76.

2. REFERENCES.

a. Report, USAEHA-LT, this Agency, Development of An Efficient Test System for Assessing Behavioral Effects of Exposure to Chemical Compounds, Study No. 51-051-73/75, November 1972 - November 1973.

b. Report, USAEHA-LT, this Agency, Preliminary Assessment of Relative Toxicity of Several Pesticide Compounds in Behavioral Screening Tests, Study No. 51-051-73/75, June 1973 - January 1974.

c. Report, HSE-LT/WP, this Agency, Preliminary Assessment of the Acute Toxicity of Malathion in Animals, Study No. 99-002-74/76, September 1973 - August 1975.

d. Report, HSE-LT/WP, this Agency, Behavioral and Biochemical Effects of 4-Benzothiethyl-N-Methylcarbamate (Mobam[®]), Study No. 51-051-73/76, June - September 1975.

3. PURPOSE. The purpose of this study was to acquire further information concerning the toxic effects of low dosages of malathion [O,O-dimethyl-S-(1,2-dicarbethoxyethyl) phosphorodithioate] on animal behavior and to compare these effects with changes in erythrocyte, plasma, and brain cholinesterase activity following treatment. This information will facilitate the evaluation of the potential toxic hazard resulting from exposure to low levels of this compound.

4. BACKGROUND. A number of organophosphorus and carbamate insecticides have been shown to disrupt the performance of learned behavior.^{1 2 3 4 5} There is some evidence that the degree of behavioral disruption may be related to the inhibition of cholinesterase (ChE) activity following exposure to these compounds. For example, in a recent report from this Agency (reference paragraph 2d), the suggestion was offered that with the experimental carbamate insecticide, Mobam[®], the inhibition of blood ChE activity may be

1 2 3 4 5 See Appendix

[®] Mobam is a registered trademark of Mobile Oil Company, New York, NY. Use of trademarked name does not imply endorsement by the US Army, but is used only to assist in identification of a specific product.

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related to one type of behavioral disorder, while decreases in central nervous system ChE activity may contribute to others. The results of some recent studies suggest, however, that this behavioral-biochemical relationship may not hold for all anticholinesterase pesticides.^{6 7 8} This relationship is of interest from a toxicological standpoint because it is often assumed that if clinical measures of ChE activity are within normal ranges after human exposure then no serious threat to health or safety exists. If ChE activity and behavioral effects are dissociated, however, this assumption may lead to the oversight of a potentially dangerous level of behavioral toxicity. The present study examined some of the behavioral and biochemical effects of the organophosphate insecticide, malathion, a compound employed extensively in both military and civilian pesticide applications. The principal area of interest was the relationship between the behavioral and anticholinesterase effects of malathion.

5. PROCEDURE.

a. Technical grade malathion, with a stated purity of 95 percent, was obtained from American Cyanamid Company, Princeton, NJ and diluted with commercial corn oil. All injections were intraperitoneal and adjusted to 1.0 ml/kg volume.

b. Male Sprague-Dawley albino rats (Wistar-derived strain) were obtained from the US Army Environmental Hygiene Agency (USAEEHA) colony. The mean body weight was 305 ± 20 g. The rats were housed individually with free access to food and water. Each experimental group consisted of ten animals.

c. The behavioral measures employed were conditioned avoidance performance and spontaneous motor activity. This provided an index of malathion effects on both learned and unlearned behavior, respectively.

(1) Conditioned Avoidance Performance. The avoidance training method followed that used in earlier studies by this Agency (reference paragraphs 2a, b, and d). The apparatus consisted of a small translucent plexiglass start box (9 cm x 12 cm x 21 cm) which opened into a larger (28 cm x 26 cm x 24 cm) cardboard-lined safe compartment by means of a portal (8 cm x 8 cm) centered on one long side of the box. Training took place the day before injection and consisted of four shock-escape trials followed by two avoidance trials, each separated by 5-minute intervals. A 1000 Hz 80 db tone sounded from the beginning of each trial until an avoidance or escape response occurred. During the escape phase of the training, the hinged top of the start box was lifted and the animal placed inside facing a side wall. This was followed immediately by tone onset and the simultaneous delivery of 1.75 mA scrambled foot shock to the grid floor of the start box. When the animal had passed over the threshold to the safe compartment it was removed and returned to its cage. The avoidance trials which followed escape training were similar except that the animal was allowed 20 seconds to leave the

^{6 7 8} See Appendix

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start box before foot shock onset. On the next day, animals were given a preliminary avoidance trial. Rats failing to avoid shock during this trial were rejected from the study and replaced. The remaining rats were injected with 25, 50, 100 or 150 mg/kg malathion or the corn oil control (0 mg/kg) and tested at one of four intervals after injection: 15 minutes, 1 hour, 4 hours or 24 hours. The dependent measure of avoidance performance was the latency to cross from the start to the safe compartment during the test trial.

(2) Spontaneous Motor Activity. Individual rats were placed into an actophotometer (Lehigh Valley Electronics Model 1497; 61 cm diameter x 42 cm height) for 5 1/2 minutes. The first 30 seconds of each test was considered an accommodation period and activity was not measured during this interval. The total number of photobeam interruptions accumulated during the subsequent 5-minute period was recorded as the activity score. Motor activity tests were conducted either 15 minutes or 1 hour after injection with 0, 25, 50, 100 or 150 mg/kg.

d. The biochemical data were obtained from the animals which had been tested in the avoidance situation. Following the post-injection avoidance test, approximately 1 ml of blood was withdrawn by intracardiac puncture for erythrocyte and plasma ChE activity analyses. The animal was then decapitated and the brain removed and frozen. Five-tenths g samples were later taken from the cerebral cortex and used for the assessment of brain cholinesterase activity. A colorimetric method, similar to that described by Levine, Scheidt, and Nelson⁹ was used and the results expressed in Garry and Routh¹⁰ units.

e. All injections, behavioral tests, and biochemical analyses were performed "blind", that is, without knowledge of which dose levels were given. Representatives from all dosage groups were randomly interspersed during the behavioral testing so that no systematic differences in order or time of test existed among the groups.

6. FINDINGS.

a. General. Clinical symptoms of toxicity (tremor, twitching) were observed in one of the animals given 150 mg/kg 1 hour after injection. The appearance of the other animals was not noticeably different from that of the controls.

⁹ J. B. Levine, R. A. Scheidt and V. A. Nelson, "An Automated Micro Determination of Serum Cholinesterase," In: Automation in Analytical Chemistry (New York: Technicon Symposium, 1966)

¹⁰ R. V. Garry and J. I. Routh, "A Micro Method for Serum Cholinesterase," Clinical Chemistry, 11, 91 (1965)

b. Avoidance. The median pre-injection avoidance latency for all groups was 0.7 sec (intraquartile range 0.5-1.0). Of the 200 tested, one animal failed to avoid shock during the preinjection test. The median postinjection latencies are shown in Table 1. As the table indicates, the latencies of the groups injected with 50, 100, and 150 mg/kg were significantly different from control (0 mg/kg) values 1 hour after injection. No significant differences were observed at the other postinjection intervals.

c. Motor Activity. The mean spontaneous motor activity scores following malathion injection are shown in Table 2. A two-way analysis of variance indicated a significant time (15 minutes versus 1 hour) effect, $F(1,90) = 13.88$, $p < .01$, but no significant dose, $F(4,90) = 1.53$, $p > .05$ or interaction $F(4,90) = 1.54$, $p > .05$ effects. Thus, it appears that while motor activity was generally greater when testing occurred shortly after injection, there was no significant dose effect of malathion on spontaneous motor activity.

d. Cholinesterase Activity. The mean erythrocyte, plasma and brain cholinesterase values are shown in Tables 3, 4, and 5, respectively. Analyses of variance indicated significant dose effects with all three measures ($F(4,180) = 27.24$, 10.62, and 7.09, respectively, all $p < .01$). The results of individual comparisons with control values within each time interval are also shown in the tables. Significant erythrocyte cholinesterase inhibition occurred 15 minutes after injection with 100 mg/kg and at 15 minutes, 1 hour, and 4 hours after injection with 150 mg/kg. Significant brain and plasma cholinesterase inhibition occurred only after 150 mg/kg, brain ChE at 15 minutes and 1 hour, plasma ChE at 4 hours.

7. DISCUSSION.

a. Table 6 summarizes the statistically significant effects observed in this study. The most important aspect of these findings is that a significant avoidance decrement was found at 50 mg/kg 60 minutes after injection without significant effects on erythrocyte, plasma, or brain ChE activity. At this dosage and time interval, the ChE measures were 91 percent, 100 percent and 100 percent of control values, respectively. A significant behavioral decrement was also found at 100 mg/kg 60 minutes after injection, again without significant ChE inhibition. It should also be noted that with both these dosages, there were no obvious clinical signs of toxicity. On the other hand, significant ChE changes were observed 15 minutes after injection with 100 mg/kg and both 15 minutes and 4 hours after injection with 150 mg/kg with no significant effects on either avoidance performance or spontaneous motor activity. These results suggest that at least some of the behavioral effects of malathion may be independent of the compound's effects on ChE activity.

TABLE 1. MEDIAN AVOIDANCE LATENCIES FOLLOWING MALATHION INJECTION

			DOSAGE MALATHION (MG/KG) *		
			0	25	50
-----15 Minutes-----					
Median (Secs)	.8	.8	.6	.7	.8
IQ Range	.6 - 3.2	.4 - 1.4	.4 - .8	.2 - .9	.3 - 1.5
P vs Control†	-	NS	NS	NS	NS
1 Hour-----					
Median (Secs)	.6	1.2	12.2	2.8	16.9
IQ Range	.4 - 1.6	.6 - 2.2	.8 - 18.8	.5 - 10.3	8.8 - 22.0
P vs Control†	-	NS	P<.02	P<.02	P<.002
4 Hours-----					
Median (secs)	.6	.7	.7	1.0	1.0
IQ Range	.5 - 1.0	.2 - 3.0	.5 - 1.2	.4 - 1.8	.8 - 3.6
P vs Control†	-	NS	NS	NS	NS
24 Hours-----					
Median (Secs)	.7	1.0	.8	.7	.9
IQ Range	.4 - 1.0	.5 - 5.2	.4 - 1.8	.4 - 1.6	.4 - 1.6
P vs Control†	-	NS	NS	NS	NS

* N = 10 for each group
 † Mann-Whitney U tests, NS (Not Significant) is given where P>.05

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TABLE 2. SPONTANEOUS MOTOR ACTIVITY FOLLOWING MALATHION INJECTION

Mean + SD % Control	Dosage Malathion (mg/kg) *			
	0	25	50	100
---15 Minutes---				
Mean + SD % Control	279 + 76 -	255 + 43 91	285 + 51 102	271 + 49 97
---1 Hour---				
Mean + SD % Control	256 + 95 -	205 + 75 80	250 + 63 98	240 + 62 94
				171 + 91 67

* N = 10 for each group

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TABLE 3. ERYTHROCYTE CHOLINESTERASE ACTIVITY (G&R UNITS) FOLLOWING MALATHION INJECTION

			DOSAGE MALATHION (MG/KG)*				
			0	25	50	100	150
15 Minutes							
Mean \pm SD	8.8 \pm 1.6		10.1 \pm 1.1	8.2 \pm 1.6	7.0 \pm 1.6	4.8 \pm 2.0	
Percent Control	-		115	93	80	54	
P vs Control†	-		NS	NS	NS	P<.05	
1 Hour							
Mean \pm SD	8.9 \pm 0.8		9.3 \pm 1.4	8.1 \pm 1.1	7.1 \pm 3.5	4.9 \pm 2.7	
Percent Control	-		104	91	80	55	
P vs Control†	-		NS	NS	NS	P<.01	
4 Hours							
Mean \pm SD	9.1 \pm 3.6		10.1 \pm 1.2	8.2 \pm 1.4	7.9 \pm 1.5	6.7 \pm 1.7	
Percent Control	-		111	90	87	73	
P vs Control†	-		NS	NS	NS	P<.01	
24 Hours							
Mean \pm SD	9.2 \pm 2.0		8.1 \pm 3.0	8.8 \pm 1.3	8.3 \pm 1.4	7.6 \pm 1.7	
Percent Control	-		88	96	90	83	
P vs Control†	-		NS	NS	NS	NS	

* N = 10 for each group

† Dunnett's t statistic comparing treatments versus control. NS is given where P>.05

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TABLE 4. PLASMA CHOLINESTERASE ACTIVITY (GER UNITS) FOLLOWING MALATHION INJECTION

		DOSAGE MALATHION (MG/KG) *					
		0	25	50	100	150	
15 Minutes							
Mean \pm SD	4.5 \pm 0.7	4.3 \pm 0.8	5.0 \pm 3.1	3.7 \pm 0.5	3.2 \pm 0.7		
Percent Control	-	96	111	82	71		
P vs Control†	-	NS	NS	NS	NS		
1 Hour							
Mean \pm SD	4.4 \pm 0.8	4.8 \pm 1.3	4.4 \pm 1.1	3.4 \pm 0.6	3.0 \pm 1.0		
Percent Control	-	109	100	77	68		
P vs Control†	-	NS	NS	NS	NS		
4 Hours							
Mean \pm SD	3.9 \pm 0.5	4.5 \pm 0.6	4.2 \pm 0.6	3.4 \pm 0.6	3.0 \pm 0.4		
Percent Control	-	115	108	87	77		
P vs Control†	-	NS	NS	NS	P<.01		
24 Hours							
Mean \pm SD	4.7 \pm 0.9	4.3 \pm 0.7	4.3 \pm 0.9	4.6 \pm 0.8	4.0 \pm 0.4		
Percent Control	-	91	91	98	85		
P vs Control†	-	NS	NS	NS	NS		

* N = 10 per group
 † Dunnett's t statistic comparing treatments vs control. NS is given where P>.05.

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TABLE 5. BRAIN CHOLINESTERASE ACTIVITY (GSR UNITS) FOLLOWING MALATHION INJECTION

		DOSAGE MALATHION (MG/KG) *			150	
		0	25	50	100	150
15 Minutes						
Mean \pm SD	64.5 \pm 14.3	53.7 \pm 13.3	69.0 \pm 14.1	58.6 \pm 13.0	41.8 \pm 07.4	
Percent Control	-	83	107	91	65	
P vs Control†	-	NS	NS	NS	P<.01	
1 Hour						
Mean \pm SD	61.9 \pm 10.6	69.3 \pm 12.9	61.6 \pm 05.0	58.9 \pm 14.5	43.9 \pm 17.9	
Percent Control	-	112	100	95	71	
P vs Control†	-	NS	NS	NS	P<.05	
4 Hours						
Mean \pm SD	58.6 \pm 10.1	61.4 \pm 13.3	61.2 \pm 10.9	63.6 \pm 11.8	54.2 \pm 15.9	
Percent Control	-	104	108	92	92	
P vs Control†	-	NS	NS	NS	NS	
24 Hours						
Mean \pm SD	56.0 \pm 13.3	54.3 \pm 09.8	62.4 \pm 11.6	63.6 \pm 11.7	59.1 \pm 14.0	
Percent Control	-	97	111	114	106	
P vs Control†	-	NS	NS	NS	NS	

* N = 10 for each group
 † Dunnett's t statistic comparing treatments vs control. NS is given where P>.05.

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TABLE 6. SUMMARY OF STATISTICALLY SIGNIFICANT DIFFERENCES IN COMPARISON WITH CONTROL GROUP VALUES

Time	Parameter	DOSAGE MALATHION (MG/KG)			
		25	50	100	150
15 Minutes	Avoidance	-	-	-	-
	Motor Activity	-	-	-	-
	Erythrocyte ChE	-	-	Eryth p<.05	Eryth p<.01
	Plasma ChE	-	-	-	-
	Brain ChE	-	-	-	Brain p<.01
1 Hour	Avoidance	-	Avoid p<.02	Avoid p<.02	Avoid p<.002
	Motor Activity	-	-	-	-
	Erythrocyte ChE	-	-	-	Eryth p<.01
	Plasma ChE	-	-	-	-
	Brain ChE	-	-	-	Brain p<.05
4 Hours*	Avoidance	-	-	-	-
	Erythrocyte ChE	-	-	-	Eryth p<.01
	Plasma ChE	-	-	-	Plasma p<.01
	Brain ChE	-	-	-	-
24 Hours*	Avoidance	-	-	-	-
	Erythrocyte ChE	-	-	-	-
	Plasma ChE	-	-	-	-
	Brain ChE	-	-	-	-

* Motor activity tests not performed at this time interval

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b. It is possible that the lack of correlation between the biochemical and behavioral measures reported here might reflect insufficient sensitivity or excessive variability in the biochemical assay procedures. However, the results of earlier work with the carbamate insecticide, Mobam, (reference paragraph 2d) using identical behavioral and biochemical procedures, suggests that for some compounds, the two types of effects are related. In fact, with this compound, erythrocyte ChE activity appeared to provide a more sensitive measure of toxicity than behavioral changes.

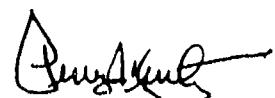
c. The absence of significant motor activity decrements after administration of dosages shown to disrupt avoidance performance is consistent with the findings of an earlier malathion study⁷ but contrasts with results obtained in this laboratory with Mobam (reference paragraph 2d). In the Mobam study, motor activity decreases were observed at dosages lower than those necessary to depress avoidance performance. The difference in findings illustrates the importance of employing more than one type of task in the assessment of behavioral toxicity.

8. CONCLUSION. From these data, it appears that malathion may disrupt rat behavior without producing significant inhibition of either blood or brain ChE activity. One must be cautious in extrapolating from animal to human exposure situations. However, these results suggest that it may be misleading to assume that behavior is normal following malathion exposure simply because blood ChE activity is within normal ranges.

9. RECOMMENDATIONS. Based on the results discussed in paragraph 6a, it is recommended that further studies be performed in order to replicate and expand upon these findings. With more information, it may be possible to identify biochemical parameters which correlate more closely with the behavioral decrements produced by malathion. In addition, it is suggested that current human screening procedures designed to monitor malathion toxicity be reviewed for their adequacy in detecting sub-clinical behavioral changes.

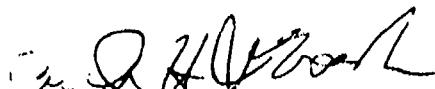
⁷ S. N. Pradhan and R. M. Mhatre, "Effects of Two Anticholinesterases on Behavior and Cholinesterase Activity in Rats," Research Communications in Chemical Pathology and Pharmacology, 1, 682 (1970)

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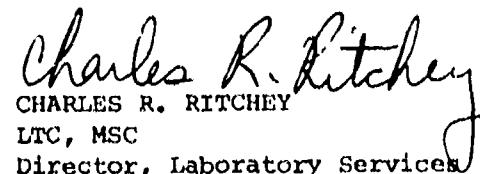


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APPENDIX

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